THE MEIOSTAGMIN REACTION

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Physicochemical reactions undoubtedly play an important part in the field of immunity. However, the finer physicochemical methods have been drawn on but little. The need of an accurate, yet simple laboratory procedure for the diagnosis of malignant disease has long been appreciated. The meiostagmin reaction was introduced to supply this need.

DEFINITION

The meiostagmin reaction (meion, small; stasso, drop) is the name given to a phenomenon which involves a lowering of the surface tension during incubation, when a diluted serum, containing certain antibodies, is mixed with its specific lipoid-containing antigen. The surface tensions of the solutions used were formerly measured by means of the Traube stalagmometer.[1]

REVIEW OF THE LITERATURE[2]

The first work on the meiostagmin reaction was done by Ascoli,[3] who determined the surface tension lowering that occurred when an alcoholic extract of typhoid bacilli, properly diluted, was mixed with a diluted typhoid immune serum, in the proportion of 1 part of the former to 9 of the latter. The surface tension was determined immediately after mixing, and again after 2 hours' incubation at 37 C. Normal serum, used in the same dilutions as the immune serum and mixed with the same alcoholic antigen, served as a control.

The alcoholic antigen was prepared as follows: A washed, agar grown culture of B. typhosus was first allowed to undergo autolysis by heating in salt solution, and was then passed through a Berkefeld filter. Part of the filtrate was evaporated to dryness, and from the residue a saturated alcoholic extract was made. This was employed as antigen in Ascoli's first work and was found to be more potent than (a) the filtrable aqueous products of autolysis, (b) the precipitate obtained by adding alcohol to (a), (c) the filtrate obtained in the preparation of (b). Various dilutions of the antigens were used, ranging from 1:100 to 1:100,000. With a 1:100 dilution of the antigens, and a 1:20 patient's serum the surface tension lowering during the 2 hours' incubation caused the drop count to increase 3.2 drops, from a stalagmometer delivering 60 drops of water. Greater dilutions of the antigens caused increases of less than

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[1] Arch. f. d. ges. Physiol. (Pflüger's Archiv), 1908, 123, p. 419.

[2] This review merely mentions the articles in chronologic order, stating in each any radical changes in recommended technic. The first two articles, however, are discussed at greater length. A more detailed review of the literature up to 1911 is given by Bernstein and Simons, Am. Jour. Med. Sc., 1911, 142, p. 852.

[3] München. med. Wchnschr., 1910, 57, p. 62.

2 drops, while control tubes containing (a) normal saline as antigen and (b) normal serum added to the typhoid extract, gave increases of one drop in each case. This work was done with a typhoid immune serum which agglutinated its specific antigen in a dilution not higher than 1:80 (macroscopically). On a basis of his experiments, Ascoli concluded that an increase of 2 or more drops warranted a diagnosis of typhoid fever.

Shortly after the appearance of Ascoli's first paper dealing with the meiostagmin reaction, one of his co-workers, Izar,[4] wrote on the same subject. Izar prepared an antigen from the spleen of a syphilitic fetus by simply extracting the desiccated tissue with absolute ethyl alcohol. The alcoholic extract obtained by repeatedly treating 5 gm. of the spleen was finally concentrated to a volume of 10 c c, and of this solution dilutions were made and employed. Specimens of serum from syphilitic patients were tested with this antigen, the dilution of the serums being 1:20, and that of the antigen 1:100. All dilutions were made with normal saline. As controls, these tests were made: (a) All serums were examined in parallel tests, but using alcoholic solutions of tubercle bacilli, carcinoma, echinococcus, and a watery solution of B. typhosus as antigens, in dilutions varying from 1:20 to 1:100; (b) nonsyphilitic serums were employed using syphilitic antigen. No nonsyphilitic serum gave an increase greater than 1 drop. In no case was there an increase of more than 1.5 drops when known syphilitic serums were tested against heterologous antigens. Two of the "Wassermann positive" serums, however did not give the usual 2 to 3 drop increase, but these were later shown to come from cases of leprosy rather than syphilis. On extending his field of investigation, Izar found that an alcoholic extract of a syphilitic liver was as potent in causing a surface tension lowering as was the alcoholic solution of spleen tissue, but that a similar extract of normal human liver, or of guinea-pig heart was not satisfactory. The work of Izar was thought to indicate, then, that the specific immune body, the so-called meiostagmin, was (a) highly specific, (b) a body of the first order (in Ehrlich's conception of immunity) and (c) that complement was not necessary for its activation, although the presence of complement did not interfere with the reaction.

In a third article by Ascoli and Izar,[5] the question of adaptability of the reaction to the diagnosis of certain malignant tumors was studied. As antigens they employed the ether soluble portion of evaporated alcoholic extracts of microscopically diagnosed tumors. Only a small percentage of the cases which were examined gave positive reactions, even when the serum of the person from whom the tumor was removed was mixed with an antigen which had been prepared from that tumor. After this preliminary investigation an easily transplantable rat sarcoma was chosen for study. The solvents used in the preparation of the antigen were the same as those mentioned. Blood serum from patients suffering from various diseases, and that of rats to which the sarcoma employed in the preparation of the last mentioned antigen had been transplanted, were all examined, in dilutions varying from 1:20 to 1:50 —the dilutions of the antigen were 1:100 and 1:500. The serums of the diseased rats caused an increase in the drop count of from 3 to 5 drops, while all other serums, including normal ones for control, gave increases of less than 1.5 drops. Antigens were prepared from 6 human carcinomas, and these, when tested against the serum of patients known to be suffering from carcinoma, gave increases in the drop count varying between 4 and 8 drops. The authors concluded that perhaps the reaction cannot be employed in the diagnosis of all tumor growths, that some of the tumors may not be suitable for

[4] Ibid., p. 182.
[5] Ibid., p. 403.

the preparation of antigens, and that the degree of surface tension change is of minor importance, just so it is greater than the experimental error, and greater than that which occurs in the control tubes.

Later, Izar and Usuelli[6] elaborated considerably on the details of the various steps in the meiostagmin reaction, and specifically stated certain essential points. I shall repeat at this point some of those which are in direct contradiction of earlier statements made by one of the authors.

(a) First count the diluted serum alone, then add the diluted antigen, incubate at 37 C. for 2 hours. This represents a variation, in that previously the first count was made immediately after addition of the antigen to the serum, rather than before this addition.

(b) The proper strength of the antigen must be arrived at by titration of various dilutions of it. Previously Izar[4] mentioned the fact that a syphilitic antigen could be used in any dilution, from 1:25 to 1:100,000, but here he insists on the necessity of a preliminary titration.

(c) An increase of two or more drops is a positive reaction. This is too broad a statement, since the number of drops varies inversely as the area of the dropping tip, and therefore the number of drops per unit volume is smaller from stalagmometers having the larger tips. It is mechanically impossible to produce suitable dropping tips with absolutely flat surfaces, and still have them all of the same diameter. In view of this possible variation in the number of drops per unit volume of the same liquid at the same temperature, and the fact that some of Izar's control tubes showed increases of 1.7 drops, the setting of an arbitrary limit of 2 drops' increase, and calling any increase above this a positive test, is unwarranted.

(d) Alcoholic extracts of normal guinea-pig hearts and of normal human liver are here reported as suitable for antigens in the diagnosis of syphilis. In a previous article Izar[4] specifically stated that these solutions were not suitable as antigens.

The serums of some of their syphilitic patients gave negative meiostagmin reactions, while in some cases positive reactions were obtained with the serum of nonsyphilitic patients. Further work was also reported by Izar and Usuelli on the diagnosis of human and rat sarcomas, in which it was shown that serums reacted not only with homologous antigens, but also with heterologous ones, although to a weaker degree.

Diluted whole blood instead of blood serum was recommended by Izar[7] in his next publication. This work dealt, for the most part, with diagnosis of certain infectious diseases, such as typhoid fever and tuberculosis. In testing serum from advanced cases of tuberculosis he found that 39 of 40 gave positive reactions. All controls were negative. Of 9 typhoid fever cases giving positive agglutination, all reacted positively with their homologous antigens. This point is mentioned here because attempts to confirm these findings failed utterly, and will be discussed later.

Ascoli and Izar[8] later immunized animals against normal horse serum, Witte's peptone, and gelatin. After several injections, the serum of these animals was able to react with antigens prepared in the usual way from these substances. These reactions were found to be specific.

After this long series of apparently brilliant results, D'Este[9] reported his findings in 19 cases of tuberculosis, 4 of them pulmonary tuberculosis, and 15 cases of surgical tuberculosis. The latter were confirmed by operation.

[6] Ztschr. f. Immunitätsf., 1910, 6, p. 624.
[7] München. med. Wchnschr., 1910, 57, p. 842.
[8] Ibid., p. 954.
[9] Berl. klin. Wchnschr., 1910, 47, p. 879.

In the pulmonary cases, the increases ranged from 1.3 to 3 drops, while in the surgical cases, the range was smaller, extending from 1 to 2.5 drops increase. In testing his antigen against normal serum, the maximum increase in the number of drops was 1.1. In twelve cases of malignant tumors, he obtained increases varying from 1 to 3 drops. It is evident from this summary of his findings that there is an overlapping in positive and control drop increases, and that the findings are not as "decidedly positive" as were those of the earlier workers.

Micheli and Cattoretti,[10] working under Ascoli, on the question of the nature of the specific substance present in the antigens which they were employing, came to these conclusions: (a) The active substances which are extracted from tumor growths, and which are responsible for the specificity of the meiostagmin reaction are lipoidal in nature, and are thermolabile; (b) not every tumor contains substances of this nature; (c) every antigen must be titrated before use in order to determine the weakest dilution that would cause an increase in the drop count amounting to 1 to 1.5 drops when mixed with diluted (1:20) normal serum. Antigens used in concentrations stronger than this react positively with normal serums also; (d) the active substances in the serums of tumor cases are relatively thermostabile.

In attempting to repeat the work of Ascoli, Verson[11] was able to secure positive results in only 10 of 18 cases of malignant tumors. Using the same antigen and serums from 6 nonmalignant cases, the readings were all negative.

Stabilini,[12] also working with malignant and nonmalignant tumors, found that of 32 cases of the former, all gave increases varying from 2.1 to 3 drops, while in 27 cases of nonmalignant growths, and other diseases, the increase never rose above 1.1 drops. He regards an increase of 1.5 to 2 drops as merely suspicious and not as a positive diagnosis.

The first attempt to employ the meiostagmin reaction in differentiating between the various types of tubercle bacilli was probably made by Gasharrini,[13] who infected rabbits and guinea-pigs with human, bovine and avian strains, and was able to obtain positive meiostagmin reactions with the serums of these animals and antigens prepared from homologous strains within a week after injection of the organisms. Working out the same plan with other organisms, Vigano[14] obtained positive reactions with the serums of 6 typhoid fever cases, using as antigens the alcohol soluble antigen prepared from suspensions of B. typhosus. The same serums, when tested against similarly prepared extracts of B. paratyphosus A and B, were all negative. I shall discuss this at a later time.

Further changes in technic were advocated in the latter part of 1910, by Ascoli and Izar.[15] The two greatest changes were (1) the preparation of antigens by extracting desiccated pulverized tumors with methyl alcohol instead of using ethyl alcohol and moist tumors, and (2) the use of water instead of normal saline in the dilution of the antigen. No reason for the change in the method of preparation of the antigen was stated. One would suppose that such changes were uncalled for in view of the uniformly good results which these and certain other workers had obtained with antigens prepared in the original way. Likewise, no reason is given for changing from normal saline to water as diluting fluid for the antigens. They specifically state that before incubation only the control tube,

[10] München. med. Wchnschr., 1910, 57, p. 1122.
[11] Wiener klin. Wchnschr., 1910, 23, p. 1102.
[12] Berl. klin. Wchnschr., 1910, 47, p. 1498.
[13] München med. Wchnschr., 1910, 57, p. 1688.
[14] Ibid., p. 1687.
[15] Ibid., p. 2129.

containing 9 c c 1:20 serum and 1 c c normal saline, is counted, and that after incubation the tube containing the solution which is being tested is counted. Since the diluent of the antigen is not isotonic with the diluent of the serum, the foregoing procedure is not scientific. The surface tension of normal saline is greater than that of distilled water, and the effect, then, of adding the former to the first solution to be tested and the latter to the one which is tested only after incubation, would be to increase the difference between the readings, or, in other words, to make a "positive result more positive."

In the same year Ascoli[16] tested the reaction in the foot and mouth disease of cattle, and obtained positive results in 22 of 28 cases, while only 2 of 35 animals which were not infected reacted positively.

Somewhat later in the same year Micheli and Cattoretti[17] were unable to verify the work of Ascoli on malignant tumors. In working with rabbits which had been immunized against B. typhosus, they were able to obtain only negative results. With syphilitic sermus, their results were positive against syphilitic antigens, but the serums from tumor cases also reacted positively with the same antigens.

Equally disappointing were the results obtained by Kelling[18] who obtained positive results with the meisostagmin reaction in only 47% of the cases of malignant tumors which came under his observation. Kelling considered any increase in drop count above 1.5 drops as positive.

Bernstein and Simons[19] never obtained what they considered a positive result. They introduced changes in technic as these were reported in the literature. Clinical cases of typhoid fever, presenting positive Widal tests, gave increases in the drop count which were no larger than those obtained by mixing the diluted patient's serums with normal saline instead of diluted antigen. Their counts were made on the solutions under test, and on the control tubes, both before and after incubation (2 hours at 37 C.). Cross tests were also made, using a known typhoid serum, a known syphilitic serum, a typhoid antigen and a syphilitic antigen. All of the tests except one showed an increase of 1 drop. This one showed a decrease of 0.5 drop. Normal salt solution, incubated in the same way, however, also showed a rise in count amounting to 1 drop. With malignant tumors their results were equally disappointing. Decided increases in the drop count were observed only when the antigens were employed in great concentration, but, as they say, "these are to be disregarded, since all investigators are agreed that antigens when used in a dilution of less than 1:50 give unreliable results, and may react strongly positive with normal serums." They conclude that in their hands the meiostagmin reaction has not proved satisfactory as a diagnostic aid.

Burmeister,[20] working with carcinomas, was unable to obtain such brilliant results as were certain of the previous workers. He experienced great difficulty in the preparation of suitable antigens.

Since the time of Burmeister's work no further work involving anything new has been reported in the literature on the question of the meiostagmin reaction. This does not mean, however, that no work has been done on this subject. One of the lamentable things in modern science is that masses of negative work remain unpublished, and that by this attitude of silence, later workers are not prevented from following "blind trails."

[16] Deutsch. med. Wchnschr., 1910, 36, p. 1997.
[17] Wiener klin. Wchnschr., 1910, 23, p. 1555.
[18] Ibid., 1911, 24, p. 90.
[19] Am. Jour. Med. Sc., 1911, 142, p. 852.
[20] Jour. Infect. Dis., 1913, 12, p. 459.

PROBLEM

In view of the failure of physicians to make use of the method of diagnosis first advocated by Ascoli and Izar, and in view of the absence of later accurate confirmatory work in the field, the feeling of doubt expressed by Zinsser [21] in these words still exists, "So far experience with the meiostagmin reaction has not been very extensive; not all observers have been able to obtain results as apparently reliable as those of Ascoli and his collaborators. It is not possible, therefore, to express a final opinion regarding this method of investigation." The questions that arise are: (a) Is the method a simple and reliable one? (b) Is the reaction specific, and if so does this specificity depend on the presence of lipoidal materials in the antigens employed? Concerning the second question, it may be stated that Kleinschmidt [22] could not produce antibodies with the streptothrix "nastin" of Much; Thiele [23] showed that lipoids possess no specificity and hence cannot give rise to antibodies. Pick and Schwarz,[24] however, found that the presence of certain lipoids increased the antigenic power of certain bacteria. This may explain why the ethereal extracts of red blood cells, used by Bang and Forsmann [25] caused the elaboration of specific hemolysins—probably by the action of the lipoids in increasing the production of antibodies, the true antigen being the traces of protein present in the extracts. It has been shown by Huntoon, Masucci and Hannum,[26] and by Krumwiede and Noble,[27] that antibodies are not lipoidal in composition. It is possible, then, that the specificity of the meiostagmin reaction, if it has such, is dependent not on the presence of extractable lipoids in the antigens employed, but rather on the protein material which may be present as "impurities."

PROCEDURE

1. *The Antigens.*—In this work a series of antigens were employed, all but two of which were extracts of agar-grown cultures of bacteria. These were numbered as indicated, and only the numbers are referred to in the tables which follow: (1) the salt solution (0.85% NaCl) soluble products of autolysis of the bacterial cells; (2) the absolute ethyl alcohol soluble portion of (1); (3) the salt solution soluble (0.85% NaCl),

[21] Infection and Resistance, 1919, p. 538.
[22] Berl. klin. Wchnschr., 1910, 47, p. 57.
[23] Ztschr. f. Immunitätsf., 1913, 16, p. 160.
[24] Biochem. Ztschr., 1909, 15, p. 453.
[25] From Jobling, Jour. Immunol., 1916, 1, p. 491.
[26] Ibid., 1921, 6, p. 185.
[27] Ibid., p. 201.

alcohol insoluble portion of (1); (4) the ether soluble portion of a mortar-ground mass of bacteria (in the preparation of this antigen petroleum ether (ligroin) B.Pt. 30-40 C. was used); (5) the acetone insoluble ether soluble portion of a bacterial mass; (6) the di-ethyl ether soluble portion of a bacterial mass; (8) the chloroform soluble portion of a bacterial mass; (10) the absolute methyl alcohol soluble portion of a bacterial mass; (11) the absolute methyl alcohol soluble portion of (1); (12) the absolute methyl alcohol soluble portion of the liver of a rabbit immunized against B. paratyphosus B. This rabbit was killed when the titer for the homologous antigen was 1:800 and that for the heterologous antigen B. suipestifer 1:400. (13) The absolute ethyl alcohol soluble portion of the same liver which was used in the preparation of (12).

Antigens 1, 2 and 3 were prepared by scraping off the growth of 20 heavily seeded agar plates of the organisms used, suspending the growth in an excess of normal saline, and washing 3 times by centrifugalization. The washed cells were again suspended in normal saline and were incubated thus for 48 hours at 37 C. to insure autolysis. The mixture was then filtered through a Berkefeld filter, evaporated to dryness, and the residue extracted as follows: for antigen 1 a saturated solution in normal saline was made; for antigen 2 a portion of the residue was extracted 3 times with warm absolute ethyl alcohol, the alcoholic extracts were combined, evaporated to dryness and the residue thus obtained was taken up in the smallest amount of alcohol that would dissolve it; for antigen 3 the alcohol insoluble residue of the evaporated products of autolysis was extracted 3 times with normal saline, the extracts were evaporated to dryness, and the residue thus obtained was taken up in the smallest quantity of normal saline necessary to dissolve it. Antigens 4, 6, 8 and 10 were all prepared from masses of bacteria which had been washed 3 times with normal saline, by centrifugalization. The only difference in the preparation of these 4 antigens was in the solvent used. The washed bacterial mass in each case was ground in a mortar with a small amount of the solvent; the mass was then extracted 3 times with the warm solvent; the combined extracts were evaporated to dryness, and the residue in each case was taken up in the smallest amount of its respective solvent that would dissolve it. Antigen 5 was prepared by dissolving in ether the acetone insoluble portion of a washed bacterial mass and concentrating the extracts as in the case of the other antigens. Antigen 11 was prepared from (1) just as (2) was, absolute methyl alcohol being used

as the solvent instead of absolute ethyl alcohol. Antigens 12 and 13 were prepared by cutting into small pieces the liver of a healthy B paratyphosus B-immune rabbit, washing the tissue with normal saline, and then extracting separate portions of it with absolute methyl and absolute ethyl alcohol, 3 times for 24 hours at 37 C. The methyl alcohol extracts were combined, evaporated to dryness, and taken up in the smallest amount of absolute methyl alcohol that would dissolve the residue. The same procedure was followed in the case of the ethyl alcohol extracts, except that in this case absolute ethyl alcohol was used as the final solvent. These antigens were employed in dilutions ranging from 1 : 10 to 1 : 10,000 as indicated in the tables which follow. All dilutions were made with physiologic sodium chlorid solution, which was prepared from chemically pure salt and triply distilled so-called "conductivity water." In all determinations 1 part of the diluted antigen was thoroughly mixed with 9 parts of the diluted blood serum. The same diluent was used for the serum as for the antigen. The surface tension of the mixture was determined before and after incubation.

2. *Obtaining the Blood Serum.*—In this work only the blood serums of rabbits were used. Four of the rabbits, 8, 9, 12 and 13, had previously been immunized against B. paratyphosus B, while 28 served as a normal control. These animals were bled from the heart by means of chemically clean all glass aspirating syringes. In order to avoid the possibilities of irregularities in readings due to possible traces of oil on the skin of the animals, the latter were first clipped closely on the left thoracic wall, and then by means of a pair of small curved dissecting scissors a small "button hole" was cut into the skin. The needle was inserted through this opening. The needles were kept in absolute ethyl alcohol when not in use. The alcohol was burned off immediately previous to the time of use. The blood was transferred from the syringe to chemically clean test tubes, in which it was first allowed to clot, and was then centrifuged. The clear serum was withdrawn with chemically clean pipets, and all dilutions were made in equally clean glass stoppered volumetric flasks of 100 c c capacity. The serums were used in dilutions ranging from 1 : 10 to 1 : 10,000.

3. *Surface Tension Apparatus.*—In this series of experiments the actual surface tension was measured in each case, and appears in the tables in dynes. Heretofore all reported work on the meiostagmin reaction was done with the stalagmometer, and the results were tabulated in "drop counts." In some work which I did almost a year ago, using alcoholic extracts of B. typhosus as antigens and diluted typhoid

immune rabbit serum as the liquid to be tested, I employed the accurate though cumbersome and slow working drop-weight apparatus [28] in my determinations. All of my results were negative, i. e., the differences between the readings before and after incubation were not greater than the limits of experimental error. Since the time consumed in making a single determination by this means varied from 50 to 90 minutes, there was reason to suppose that perhaps all possible surface tension lowering had taken place before the time of completion of the first determination—before incubation. For that reason, all determinations recorded in this paper were made with the Du Nouy tensiometer [29] which is reliable, simple, accurate, and rapid. All of my determinations were not made at the same temperature, but the surface tension was in each case reduced by calculation to what would have been at 22 C., the temperature at which most of the readings were taken. This was done so as to render the results comparable.

4. *Method of Making the Tests.*—As stated, the solutions were employed in the proportion of 1 part of the diluted antigen to 9 of the diluted serum. The surface tension was determined, after thorough mixing, and the transferring of about 2 c c of the mixture to a Syracuse watch glass, by means of a pipet. The remainder (about 3 c c in each case) of the liquid and the pipet were incubated in a water bath at 37 C. for one and a half hours. After incubation the tube and pipet were cooled to 22 C. in a water bath, and the surface tension was again determined. In order to insure the cleanliness of all the apparatus which was used, each piece was first boiled in a strong soap solution, then rinsed thoroughly in running tap water, immersed for upward of half an hour in a sulphuric acid-potassium bichromate cleaning solution, rinsed 3 times in (a) tap water, (b) distilled water, and (c) conductivity water. One of the tables indicates the effectiveness of this treatment.

At the beginning of this work it was noticed that a spontaneous decrease took place in the surface tension of blood serum-antigen mixtures when exposed to the air in the watch glasses. This was found to be due to substances in the serum, and is a property of normal serums as well as of immune serums. In fact, solutions of pure hemoglobin, or any other substance of large molecular dimensions exhibit the same phenomenon. With the serum mixtures, this decrease amounted

[28] Jour. Am. Chem. Soc., 1916, 41, p. 499; 38, p. 246.
[29] Jour. Gen. Physiol., 1919, 1, p. 521.

to from 1.4 to 2.2 dynes in the first 2 minutes, exposure. Because of these facts I always waited for from one to one and one-half minutes before taking readings, and then averaged the fourth, fifth and sixth, disregarding the first 3.

The following tables and discussion summarize the results of over 1,100 determinations which were made on almost 200 different antigen-serum mixtures, and which are characterized by one thing in particular, namely, the uniformity of negative findings with my 4 immune serums and all of my antigens.

The serums used in the tests were from rabbits that had been immunized against the specific organism from which the antigens were prepared. The titer of the serums were above 1:6,000 (macroscopic

TABLE 1.

RESULTS OF TESTS WITH SERUM OF RABBITS IMMUNIZED AGAINST THE SPECIFIC ORGANISM FROM WHICH ANTIGENS WERE PREPARED *

Antigens	Rabbit Serum 8			Rabbit Serum 12		
	Before	After	Difference	Before	After	Difference
B. paratyphosus B (1) 1:100.....	77.9	78.4	+0.5	78.2	77.9	—0.3
B. paratyphosus B (1) 1:1,000.....	78.6	78.2	—0.4	78.2	78.1	—0.1
B. paratyphosus B (1) 1:10.000.....	78.3	77.8	—0.5	78.2	77.9	—0.3
B. paratyphosus B (8) 1:100.....	69.3	68.3	—1.0	78.2	78.3	+0.1
B. paratyphosus B (8) 1:1,000.....	69.2	67.7	—1.5	78.2	78.3	+0.1
B. paratyphosus B (8) 1:10,000.....	69.6	67.7	—1.9	78.2	78.2	0.0
B. paratyphosus B (6) 1:100.....	....	....	.....	78.3	77.9	—0.4
B. paratyphosus B (6) 1:1,000.....	....	....	.....	78.3	77.8	—0.5
B. paratyphosus B (6) 1:10,000.....	....	....	.....	78.1	77.9	—0.2

* The numbers in parentheses after the name of the antigen indicate what the extractive was in each case ("Preparation of Antigens"). Next comes the dilution of the antigen, then a figure representing the average of three surface tension determinations made before the time of incubation, another figure representing the same thing for determinations made after incubation, and finally, the difference (in dynes) between these averages.

agglutination). In the reports of the Italian workers increases of over 2 drops in the drop counts were considered positive. The liquids employed were such that the drop counts were between 56 and 60 drops from a stalagmometer delivering 60 drops of water at 20 C. Since the total volume of liquid delivered was constant, and since the density was constant, the surface tension must have varied inversely as the number of drops, hence since the surface tension of water at 20 C. is about 78 dynes (28), and that of the antigen serum complex was nearly that, an increase of over 2 drops would indicate a surface tension lowering of over 1.6 dynes. On that basis all but one of the readings in table 1 are negative, and even in the case of that one, a parallel determination on the serum of rabbit number 12 was distinctly negative. The serum in the foregoing series was used in a 1:10,000 dilution.

According to a previously cited report, the antigens must be titrated before use as in the higher concentrations they will act positively even with normal serums. Table 2 contains a series of determinations which were made in an attempt to confirm this statement.

TABLE 2

RESULTS OF TESTS MADE WITH SERUMS TITRATED BEFORE USE

Serum Dilutions	Surface Tension		
	Before	After	Difference
1:10	61.8	60.5	—1.3
1:20	63.3	63.3	0.0
1:40	65.4	65.2	—0.2
1:80	65.4	65.2	—0.2
1:100	65.3	65.2	—0.1
1:1,000	75.9	75.7	—0.2
1:10,000	77.9	78.4	+0.5

* Antigen, B. paratyphosus B 1, dilution 1:10; serum from B. paratyphosus immune rabbit 8.

TABLE 3

RESULTS WITH SERUMS OF TWO RABBITS IMMUNIZED AGAINST B. PARATYPHOSUS B, AND ONE NORMAL RABBIT *

Antigens	Rabbit 8			Rabbit 12			Rabbit 28 (Control)		
	Before	After	Difference	Before	After	Difference	Before	After	Difference
B. parat. B (1) 1:20	....	....	.....	63.3	63.3	0.0	....	....	.....
B. parat. B (1) 1:20	....	....	.....	63.9	63.6	—0.3	....	....	.....
B. parat. B (1) 1:100	67.9	67.8	—0.1	67.0	67.0	0.0	66.4	66.8	+0.4
B. parat. B (10) 1:100	66.8	67.3	—0.5	66.4	67.3	+0.9	64.9	65.9	+1.0
B. parat. B (10) 1:1,000	68.3	68.7	+0.4	66.6	66.8	+0.2	65.2	67.0	+1.8
B. parat. B (11) 1:100	66.5	67.3	+0.8	65.6	66.1	+0.5	66.2	65.6	—0.6
B. parat. B (11) 1:1,000	67.0	66.3	—0.7	66.4	66.7	+0.3	65.6	65.8	+0.2
B. typh. Hop. (2) 1:100	66.2	68.2	+2.0	64.7	64.6	—0.1	....	....	.....
B. typh. Bari. (2) 1:1,000	67.8	68.0	+0.2	64.7	64.6	—0.1	66.4	66.7	+0.3
B. dys., Flex. (2) 1:100	67.4	67.7	+0.3	64.2	64.2	0.0	67.7	66.1	—1.6
B. dys., Shig. (2) 1:100	67.7	66.8	—0.9	64.1	63.6	—0.5	....	....	.....
B. suipestifer (2) 1:100	68.3	68.3	0.0	63.1	63.1	0.0	66.7	66.7	0.0
B. suipestifer (4) 1:100	67.4	67.2	—0.2	64.9	63.4	—1.5	67.4	66.4	—1.0
B. suipestifer (6) 1:100	67.3	66.3	—1.0	63.0	62.8	—0.2	....	....	.....
Control. salt solution plus dil. serum	69.9	68.9	—1.0	....	....	.....	....	....	.....

* In table 3 and in those which follow: B. parat. B stands for B. paratyphosus B strain 12, B. typh. Hop. for B. typhosus (Hopkins), B. typh. Bari. for B. typhosus (Barimore), B. dys., Flex. for B. dysenteriae (Flexner), B. dys. Shig. for B. dysenteriae (Shiga) and B. suipestifer 118 is represented by B. suipest.

There is nothing in this series of determinations to verify the statements referred to.

The results recorded below in table 3 were obtained with 1:20 diluted serums of 2 rabbits which had been immunized against B. paratyphosus B and with 1 normal rabbit, 28, which served as a control. In this series antigens prepared from the organism used in the immuniza-

tion of the rabbits, and, in addition, several other heterologous antigens, were employed. Included in the heterologous series were some prepared from B. suipestifer, which was agglutinated by the paratyphoid immune serums in almost as high dilutions as was the homologous organism. The dilutions and numbers of the various antigens are recorded as they were in table 1.

Determinations with the same antigens and the serums (1:20) of B. paratyphosus-immune rabbits 9 and 13 are not recorded. The results were similar to those tabulated in the foregoing. Serums from the same 5 animals were employed in dilutions of 1:100 and 1:1,000 mixed with homologous antigens 1, 2, 4, 6 and 8 and with the heterologous ones which are recorded in table 3. The antigens were

TABLE 4

RESULTS OF TESTS WITH ANTIGENS 12 AND 13. DILUTION OF SERUMS 1:20 IN ALL CASES

Antigens	Rabbit 8			Rabbit 9			Rabbit 12		
	Before	After	Difference	Before	After	Difference	Before	After	Difference
B. parat. B (12) 1:100	63.4	64.9	+1.5	64.6	64.2	—0.4	64.4	63.3	—1.1
B. parat. B (12) 1:1,000	64.6	66.0	+1.4	61.9	62.9	+1.0	64.6	64.9	+0.3
B. parat. B (13) 1:100	64.4	65.0	+0.6	63.9	62.0	—1.9	66.5	66.9	+0.4
B. parat. B (13) 1:1,000	66.7	66.3	—0.4	64.3	65.5	+1.2	65.8	65.5	—0.3
	Rabbit 13			Rabbit 28 (Control)					
B. parat. B (12) 1:100	62.8	63.9	+1.1	63.5	63.9	+0.4			
B. parat. B (12) 1:1,000	65.9	64.3	—1.6	....	....	.....			
B. parat. B (13) 1:100	63.5	64.9	+1.5	65.6	65.9	+0.3			
B. parat. B (13) 1:1,000	65.8	64.9	—0.9	65.4	65.1	—0.3			

used in dilutions of 1:100 and 1:1,000 when mixed with the homologous serums and 1:100 only in the cases of the typhoid, dysentery and suipestifer antigens. The results of these experiments are not recorded because the differences and small irregularities which were observed were similar in every respect to those of table 3.

This work fails in every way to confirm the findings of the earliest workers with the meiostagmin reaction. Only occasionally were results obtained which were even "suspicious," but other tests in parallel series failed to check up with these cases or normal serums, to which the same antigens had been added, or to which (in two cases) only normal saline had been added, caused equally large changes. The limits of experimental error are as great in the higher dilutions of antigen and serum as in the more concentrated mixtures.

After this long series of disappointingly negative experiments, it was decided to make one final effort to obtain a positive result. A rabbit which had been immunized against B. paratyphosus B, and whose serum agglutinated the homologous antigen in a 1:800 dilution, was given an overwhelmingly large injection of a live culture of this organism. The animal died within 6 hours. The liver was removed, and from it antigens 12 and 13, discussed in the foregoing, were prepared. These

TABLE 5

DETERMINATIONS ON "CONDUCTIVITY WATER," SODIUM CHLORIDE SOLUTION AND SALT SOLUTION PLUS ANTIGEN

	Before			After			Difference
Determinations on "Conductivity Water"							
Sample 1	105.1	105.2	105.1 = 105.1 = 77.9	105.0	104.9	105.1 = 105.0 = 77.8	—0.1
Sample 2	105.1	105.3	105.4 = 105.3 = 78.1	105.2	105.1	105.1 = 105.1 = 77.9	—0.2
Sample 3	105.1	105.1	105.1 = 105.1 = 77.9	105.1	104.9	105.0 = 105.0 = 77.8	—0.1
Determinations on 0.85% Sodium Chloride Solution							
Sample 1	105.7	105.7	105.7 = 105.7 = 78.3	105.6	105.4	105.7 = 105.6 = 78.3	0.0
Sample 2	105.4	105.3	105.4 = 105.4 = 78.2	105.4	105.4	105.4 = 105.4 = 78.2	0.0
Sample 3	105.6	105.6	105.4 = 105.5 = 78.3	105.5	105.7	105.5 = 105.6 = 78.3	0.0
Determinations on Salt Solution Plus Antigen (In each of the determinations which appear in this section, 9 parts of normal saline were mixed with one part of diluted antigen)							
Sample 1, salt plus B. parat. B (2) 1:100	105.3	105.2	105.3 = 105.3 = 78.1	105.3	105.3	105.3 = 105.3 = 78.1	0.0
Sample 2, salt plus B. parat. B (4) 1:100	105.5	105.6	105.4 = 104.5 = 78.2	105.4	105.3	105.3 = 105.3 = 78.1	—0.1
Sample 3, salt plus antigen B. parat. B (6) 1:100	105.3	105.4	105.3 = 105.3 = 78.1	105.3	105.4	105.3 = 105.3 = 78.1	0.0
Sample 4, salt plus antigen B. parat. B (8) 1:100	105.8	105.7	105.8 = 105.8 = 78.4	105.8	105.7	105.8 = 105.8 = 78.4	0.0
By adding 1 c c of normal saline to 9 c c of a 1:20 dilution of rabbit serum the following differences were noted							
	Before Incubation			After Incubation			Difference
Rabbit 9	88.4	87.9	87.9 = 88.1 = 65.4	86.0	85.6	85.4 = 85.7 = 63.7	—1.7
Rabbit 13	88.9	88.0	88.0 = 88.3 = 65.5	88.4	88.0	87.8 = 87.7 = 65.2	—0.3
Rabbit 28	89.4	89.0	88.9 = 89.1 = 66.1	89.8	89.4	89.1 = 89.4 = 66.4	+0.3

antigens were tested against the potent B. paratyphosus B. immune serums of rabbits 8, 9, 12 and 13, and against the normal serum of control rabbit 28. The results of this work are recorded in table 4.

The results here tabulated are more erratic in appearance than those in the earlier tables. The peculiar thing is that deviations occur in both directions, i. e., some indicate increases in the surface tension, while others show decreases. The results are therefore as strikingly negative as those obtained with the other antigens, and correspond to

the findings of Bernstein and Simons,[19] who used positive typhoid and syphilitic serums and typhoid and syphilitic antigens in a series of cross tests. In their work, normal salt solution gave as great a decrease in the surface tension as their antigen-serum mixtures did, and their syphilitic antigen syphilitic serum mixture gave no decrease, while a syphilitic serum typhoid antigen mixture gave an increase.

Because of the irregularity with which surface tension lowering was observed, and also because at times a lowering was observed in one tube while a similar tube in a parallel series showed an actual increase, a set of control tests was run in order to determine these points: (a) the accuracy of the instrument; (b) the presence or absence of impurities on the glassware used; (c) the surface tension changes taking place in pure water and in normal saline during a period of incubation similar to that used in the actual tests; (d) which substances were responsible for the irregularities observed in the antigen-serum mixtures?

The table shows readings as taken in triplicate, the average of the 3, and the surface tension corresponding to this average, both before and after incubation, and the difference if there was any.

From these sets of controls it is evident that the apparatus gives accurate readings, the cleaning of the glassware is adequate, pure water, salt solution, and diluted antigen alone do not give irregular spontaneous surface tension changes during incubation, and that it is the serum which is responsible for the large experimental error.

SUMMARY AND CONCLUSIONS

Over 1,100 tests were made in a study of the meiostagmin reaction, using almost 200 different mixtures of rabbit serum and antigen. The serum was obtained from animals immunized against B. paratyphosus B, and the antigens were prepared from the homologous and several heterologous organisms, and from the liver of a healthy B. paratyphosus B immune rabbit.

The spontaneous surface tension changes and the limits of experimental error are as great when relatively dilute serum is employed in the test as when the serum is diluted only 1 : 20. Serum more dilute than 1 : 1,000 shows a smaller degree of, and a slower rate of, spontaneous surface tension change than lower dilutions. These facts apply to normal as well as immune serums.

The substance in the antigen-serum mixtures which is responsible for relatively large experimental error is the blood serum, as measurements on the various other substances used in these mixtures gave constant and accurate readings.

The Du Nouy surface tension apparatus, not previously commented on by any one but its inventor, gives readings with the biochemical mixtures employed, which are as accurate as those obtainable with the more cumbersome and slow drop weight apparatus. The sources of experimental error are less than those involved in the use of the Traube stalagmometer.

The meiostagmin reaction does not reveal the presence of antibodies in B. paratyphus B immune rabbit serum of high titer regardless of (a) the dilutions in which the serums and antigens are employed, and (b) of the solvents used in the preparation of the antigens.